IS 11479-1 (2001): Antibacterial Toilet Soap, Part 1: Solid Cake [CHD 25: Soaps and other Surface Active Agents]

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भारतीय मानक

जीवाणु रोधी प्रसाधन साबुन — विशिष्टि

भाग 1 ठोस बट्टी

(पहला पुनरीक्षण)

Indian Standard

ANTIBACTERIAL TOILET SOAP — SPECIFICATION

PART 1 SOLID CAKE

(First Revision)

ICS 71.100.40

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

August 2001

Price Group 5

FOREWORD

This Indian Standard (Part 1) (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Soaps And Other Surface Active Agents Sectional Committee had been approved by the Chemical Division Council.

Human skin provides a favourable environment for the existence and multiplication of a variety of microbes. The conventional toilet soap washes away the germs but does not kill them. The function of an antibacterial or antiseptic toilet soap is not only to clean the skin, but also to reduce drastically the bacterial count on the skin. This prevents skin infections and perspiration odour caused by the decomposition of perspiration by bacteria.

The antibacterial toilet soap is specially effective against *staphylococcus* and similar bacteria which have the habit of residing in the under layers of skin. The antibacterials are substantive to the skin and this tackles the microbes between two washes. Antibacterial toilet soaps shall be used regularly to be effective.

In the present revision hexachloroprene has not been permitted to use as antibacterial agent. Trichlorocarcanilide (TCC) on heating decomposes to chloroanilines which can be harmful to skin and hence the limit and method for determination of chloroaniline is incorporated. It is decided to publish this standard as Part 1(solid cake) and antibacterial liquid toilet soap will form Part 2.

A scheme for labelling environment friendly products known as ECO-Mark has been introduced at the instance of the Ministry of Environment and Forests (MEF), Government of India. The ECO-Mark would be administered by the Bureau of Indian Standards (BIS) under the *Bureau of Indian Standards* Act, 1986 as per the Resolutions No. 71 dated 21 February 1991 and No. 425 dated 28 October 1992 published in the Gazette of the Government of India. For a product to be eligible for marking with ECO logo, it shall also carry the ISI Mark of BIS besides meeting additional environment friendly requirements. The requirements to be satisfied for a product to qualify for the BIS Standard Mark for ECO friendliness, has been included in this revision. These requirements will be optional; manufacturing units will be free to opt for the ISI mark alone also.

The requirements of the conventional grade of toilet soaps are given in IS 2888 : 1983 'Specification for toilet soap (*second revision*)'.

There is no ISO specification on this subject. This standard is formulated based on indigenous technology and data available.

The composition of Committee responsible for formulating this standard is given in Annex D.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

AMENDMENT NO. 1 SEPTEMBER 2008
TO
IS 11479 (PART 1) : 2001 ANTIBACTERIAL TOILET SOAP — SPECIFICATION

PART 1 SOLID CAKE

(First Revision)

(*Foreword, para* 1) — Insert the following after para 1:

"IS 11479 : 1985 'Antibacterial toilet soap' was published in 1985. During the revision, the Committee felt that it would be more convenient to split the standard into two parts. Part 1 covered Solid cake and Part 2 covered Liquid.

This standard supersedes IS 11479 : 1985 'Antibacterial toilet soap'."

(CHD 25)

Reprography Unit, BIS, New Delhi, India

Indian Standard

ANTIBACTERIAL TOILET SOAP — SPECIFICATION

PART 1 SOLID CAKE

(First Revision)

1 SCOPE

This standard (Part 1) prescribes the requirements and methods of sampling and test for antibacterial toilet soap, solid cake.

2 REFERENCES

The Indian Standards listed below contain provisions which through reference in this text, constitute provisions of this Indian Standard. At the time of publication, the editions indicated were valid. All standards are subject to revisions, and parties to agreements based on this Indian Standard are encouraged to investigate the possibility of applying the most recent editions of the Indian Standards.

IS No.	*Title*
286 : 1978	Methods of sampling and test for soaps (*second revision*)
1070 : 1992	Reagent grade water (*third revision*)
4955 : 1993	Household laundry detergent powders (*third revision*)
7597 : 1974	Glossary of terms related to surface active agents
13424 : 1992	Safety evaluation of bathing bar and toilet soap — Methods of test

3 TERMINOLOGY

For the purpose of this standard, the definitions given in 2 of IS 286 and IS 7597 shall apply.

4 REQUIREMENTS

4.1 Description

Antibacterial toilet soap shall be a high grade, thoroughly saponified, milled soap or homogenized soap or both, white or coloured, perfumed, and compressed in the form of firm and smooth cakes, and shall possess good cleaning and lathering properties.

4.2 Ingredients

In addition to perfume, moisture, normal colouring matters, preservatives acceptable in toilet soaps in general, the antibacterial soap shall contain permitted antibacterial agent (*see* **4.2.1**). The label shall clearly state the antibacterial agent used and its level. The soap shall pass the antibacterial activity test when determined by the method given in Annex A.

4.2.1 Triclosan (TCN) and Trichlorocarbanilide (TCC) shall not exceed 1 percent by mass either singly or in combination, when tested by the method prescribed in Annex B .

4.2.2 Chloroaniline content shall not exceed 10 ppm when tested by the method prescribed in Annex C.

NOTE — TCC is not heat stable and decomposes into chloroanilines on prolonged heating above 60°C. If TCC is used in soap, the manufacturer should take care that such soap is not subjected to temperature above 60°C during the entire manufacturing process or during storage.

4.3 Antibacterial toilet soap, solid cake shall also comply with the requirements specified in Table 1.

Table 1 Requirements for Antibacterial Toilet Soap, Solid Cake

Sl No.	Characteristic	Requirements	Test Method (Ref to Cl No. in IS 286)
(1)	(2)	(3)	(4)
i)	Total fatty matter, percent by mass, *Min*	76.0	15
ii)	Rosin acids, percent by mass, of total fatty matter, *Max*	3.0	14
iii)	[1]Free caustic alkali, as sodium hydroxide (NaOH), percent by mass, *Max*	0.05	6.2
iv)	[1]Free carbonated alkali, as sodium carbonate (Na_2CO_3), percent by mass, *Max*	1.0	28
v)	[1]Matter insoluble in alcohol, percent by mass, *Max*	2.5	5

[1] *See also* **4.3.1**.

4.3.1 *Calculation of Results*

Antibacterial toilet soap is liable to lose moisture on keeping. The results of analysis in respect of free caustic alkali, free carbonated alkali and matter insoluble in alcohol shall be recalculated in relation to the minimum specified total fatty matter by means of the following equation:

$$\text{Recalculated result} = \text{Actual result} \times \frac{\text{Minimum specified total fatty matter}}{\text{Actual total fatty matter}}$$

4.3.2 The antibacterial toilet soap shall pass the test for dermatological safety when evaluated as per the method prescribed in IS 13424.

4.4 Additional Requirements for ECO-Mark

4.4.1 *General Requirements*

4.4.1.1 The product shall conform to the requirements for quality, safety and performance prescribed under **4.1** to **4.3**.

4.4.1.2 The manufacturer shall produce to BIS environmental consent clearance from the concerned State Pollution Control Board as per the provisions of *Water (Prevention and Control of Pollution) Act*, 1974 and *Air (Prevention and Control of Pollution) Act*, 1981 along with the authorization, if required, under the *Environment (Protection) Act*, 1986 while applying for ECO-Mark.

4.4.2 *Specific Requirements*

4.4.2.1 The antibacterial toilet soap shall neither contain any synthetic detergent when tested as per the method given in Annex B and C of IS 4955 nor any phosphate when tested as per the method prescribed in **20** of IS 286.

4.4.2.2 The antibacterial toilet soap shall pass the test for dermatological safety when evaluated as per the method prescribed in IS 13424.

5 PACKING AND MARKING

5.1 Packing

The product shall be packed as agreed to between the purchaser and the supplier.

5.1.1 For ECO-mark the product shall be packed in such packages which are made from recyclable/ reusable or biodegradable material and declared by the manufacturer and may be accompanied with detailed instructions for proper use.

5.2 Marking

The packages shall be securely closed and marked with the following particulars:

a) Indication of source of manufacture;
b) Brand name of the material and recognized trade mark, if any;
c) Net mass when packed;
d) Batch No. or lot No. in code or otherwise;
e) Month and year of manufacture; and
f) The following identified critical ingredients in descending order of quantity; percent by mass.
   1) Total fatty matter (TFM),
   2) Matter insoluble in alcohol, and
   3) Antibacterial agent.

5.2.1 *Additional Information for Eco-Mark*

The criteria for which the product has been labelled as ECO-Mark.

5.2.2 *BIS Certification Marking*

The packages may also be marked with the BIS Standard Mark. The use of the Standard Mark is governed by the provisions of *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

6.1 For this purpose general precautions, scale of sampling and preparation of test samples shall be as prescribed in **3** of IS 286.

6.2 Number of Tests

6.2.1 Tests for determination of total fatty matter and free caustic alkali and matter insouble in alcohol shall be conducted on each of the individual samples separately.

6.2.2 Tests for determination of all the remaining characteristics shall be conducted on the composite sample.

6.3 Criteria for Conformity

6.3.1 For each of the characteristics which has been determined on the individual samples (*see* **6.2.1**) the mean ($\bar{X}$) and the range (R) of the test results shall be calculated as follows:

$$\text{Mean } (\bar{X}) = \frac{\text{sum of test result}}{\text{number of test result}}$$

Range (R) = The difference between the maximum and the minimum value of test results.

The lot shall be deemed as conforming to the requirements given in **6.2.1** if the expression ($\bar{X} - 0.6\,R$) is greater than or equal to minimum value given in Table 1 and ($\bar{X} + 0.6\,R$) is less than or equal to maximum value given in Table 1.

6.3.2 For declaring the conformity of a lot to the requirements of other characteristics determined on the composite sample, the test results for each of the characteristics shall satisfy the relevant requirement.

7 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in the tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A

(*Clause* 4.2)

DETERMINATION OF ANTIBACTERIAL ACTIVITY

A-1 GENERAL

Two methods have been prescribed, namely, serial dilution method and substantivity test. The serial dilution test shall be the screening test and the substantivity test shall be the absolute test.

A-2 SERIAL DILUTION TEST

A-2.1 Outline of the Method

Antibacterial activity is determined by serial dilution method by comparing the effectiveness of antibacterial chemicals present in 10 micrograms of soap per milliliter specified as the maximum inhibitory concentration.

A-2.2 Apparatus

A-2.2.1 *Culture Tube, Rimless* — 150 × 18 mm.

A-2.2.2 *Sterilized Pipettes* — 10 ml, 5 ml and 1 ml capacities.

A-2.2.3 *Loop Made of Stainless Steel or Platinum Wire*

A-2.2.4 *Conical Flasks* — 250 ml capacity.

A-2.3 Reagents

A-2.3.1 *Nutrient Broth*

A-2.3.1.1 Dissolve 5 g of beef extract, 5 g of sodium chloride, 10 g of peptone in one litre of distilled water by warming over a water bath. Cool and adjust the *p*H to 7.2 to 7.6 with sodium hydroxide solution. Distribute 9 ml each to the culture tubes. Plug the tube with non-absorbent cotton wool and sterilize in an autoclave for half an hour at 1 kg/cm^2 pressure.

A-2.3.1.2 Take 99 ml and 90 ml of distilled water in 250-ml conical flask. Plug them with non-absorbent cotton wool and sterilize in an autoclave.

A-2.3.1.3 Get a pure stain of *staphylococcus aureus ATCC 6538P*. Maintain on nutrient agar medium. Transfer to a fresh slant every month and keep in the cold. Use a 24 hours nutrient broth culture for the experiment.

A-2.4 Procedure

A-2.4.1 Aseptically transfer 1 g of the soap sample to the flask containing 99 ml of water. Dissolve by slight warming not exceeding 60°C. Transfer 10 ml of this solution to another flask containing 90 ml of water. Take 1 ml of this solution and add 9 ml of nutrient broth in a culture tube. This gives a concentration of 100 µg/ml.

A-2.4.2 To three tubes containing 9 ml nutrient broth add 1 ml each of the above solution to get a concentration of 10 µg of soap per ml of nutrient broth in each tube. Inoculate the tubes with a loopful of the 24 hours culture of *staphylococcus aureus* and keep them in an incubator maintained at 37 ± 2°C. Keep a control tube of nutrient broth containing the same concentration of soap.

A-2.4.3 If after 24 h incubation period, the liquid in all the three tubes is as clear as the control, the soap sample passes the test. Any turbidity more than the control shows the growth of bacteria.

A-3 SUBSTANTIVITY TEST

A-3.1 Basic Principles

For a soap to have antibacterial activity, it shall satisfy two criteria:

a) It shall show, antibacterial activity on the skin even after the soap is rinsed away, that is, the germicide should be retained on the skin under the conditions of use.

b) The antibacterial activity should be retained on the skin for some period so as to provide protection to the skin.

A-3.2 The test devised gives a measure of both these properties. The test involves application of soap solution on the forearm, rinsing it off in running water and allowing it to dry. A mixed culture of skin flora isolated from 5 individuals (*see* **A-3.3.1**) is applied immediately in prescribed areas and assayed by swabbing at 0 and 10 min. The percent reduction in survivors in 10 min is determined. Similarly the soap solution after rinsing is allowed to remain on the skin for 2 h. The test micro-organisms are applied to the skin at this time in prescribed areas and assayed by swabbing at 0 and 10 minutes. The percent reduction in survivors is determined. If the reduction in survivors at this time is greater than 45 percent, the germicide is said to be substantive.

A-3.3 Method

A-3.3.1 *Test Micro-organisms*

The test organisms, consist of a mixed skin flora, prepared by collecting washings from the arms and forearms of at least 5 individuals using 50 ml of sterile water in each case. Ten ml aliquot of each washing is individually inoculated into flasks containing 90 ml of sterilized nutrient broth. Culture is allowed to grow

overnight at 30°C and flasks showing turbidity are pooled together. The mixed culture is transferred through broth and grown as above at least 3 times and finally maintained as Tryptone-Agar-Glucose Yeast Extract (TGYE) agar, Trypticase Soy Agar (TSA), Nutrient Agar (NA) or similar agar slants. For a test culture, an overnight slant culture is suspended into sterile saline and adjusted to a cell population of 1×10^7 cells per ml.

A-3.3.2 *Test Procedure*

A-3.3.2.1 A number of 4 cm^2 areas (2 × 2 cm) are marked out on the innerside of the forearm. 0.1 ml aliquot of an 8 percent soap solution with germicide is applied onto individual squares and allowed to dry for 1 minute. The areas are then washed with a gentle flow of tap water for two minutes, dried by blowing warm air. The retentivity of the germicide on skin and its antibacterial action are then assayed by applying 0.1 ml of mixed skin flora (10^7 cells/ml) on to 4 such squares at 0 h. Two of the squares are swabbed immediately using standard sterile cotton swabs on a stick. Swabs are placed in 5 ml saline solutions. Contents are shaken well in a vortex mixer and ten fold dilutions are prepared. Bacterial cells are assayed on TGYE agar, TSA or NA plates to determine the initial count. After 10 minutes, two other squares are swabbed and assayed in a similar manner.

A-3.3.2.2 In another set of tests, soap solutions are applied to the 4 more squares, dried and rinsed. After allowing 2 hours interval, 0.1 ml of culture is applied as above to 4 squares. Two of the squares are swabbed and assayed at 0 h and remaining two after 10 minutes. Survivals at 0 h and after 2 h are determined.

A-3.4 The soap shall be considered to have passed the test if the percent kill is greater than or equal to 45 percent after 2 h challenge.

ANNEX B

(*Clause* 4.2.1)

DETERMINATION OF TCC AND TCN IN SOAPS BY HPLC

B-1 PRINCIPLE

TCC and TCN are antibacterial agents, which are separated from other components in soap by normal phase or reverse phase liquid chromatography, detected spectrophotometrically and quantified by comparison with standard TCC and TCN. The method can estimate as low as 1 ppm of the above compounds.

Procedures for both normal and reverse HPLC has been described and provide the option to use either method whichever is available to the users. Both methods are comparable.

B-2 NORMAL PHASE HPLC

B-2.1 Reagents

B-2.1.1 *Iso-octane* — HPLC grade.

B-2.1.2 *Iso-propanol (2-propanol)* — HPLC grade.

B-2.1.3 *Hexane* — HPLC grade.

B-2.1.4 *Standard TCC* – 99 percent pure .

B-2.1.5 *Standard TCN* — 99 percent pure.

B-2.2 Apparatus

B-2.2.1 *High Performance Liquid Chromatograph* — Consisting of a pump, a sample injector of fixed volume with UV detector having variable wavelengths and a recorder.

B-2.2.2 *Standard Volumetric Flasks*

B-2.2.3 *Pipettes*

B-2.2.4 *Magnetic Stirrer*

B-2.2.5 *Millipore Filter Apparatus with 0.5 μ Filter*

B-2.2.6 *Column*

B-2.2.6.1 *Silica column*

Stainless steel 25 cm × 0.46 cm packed with Normal phase – Silica 5 μ (Lichrosorb Si-60)

or

B-2.2.6.2 *Cyano column*

Stainless steel 25 cm × 0.40 cm packed with (Lichrospher 100) cyano 5 μ.

NOTE — Either of the above columns can be used depending on the availability.

B-2.2.7 *Mobile Phase*

B-2.2.7.1 *For silica column* — Transfer 20 ml of *iso*-propanol into a 500 ml volumetric flash and make upto mark with *iso*-octane and mix well. Assemble milllipore filter apparatus and filter the solvent system prior to use.

B-2.2.7.2 *For cyano column* — Transfer 50 ml of HPLC grade *iso*-propanol (2-propanol) into a 500 ml volumetric flask, fill upto the mark with hexane and mix well assemble milllipore filter apparatus and filter the solvent system prior to use.

B-2.2.8 *HPLC Conditions*

Detector wavelength : 280 nm
Flow rate : 0.5 ml/min
Injection volume : 20 µl
Retention time
Silica column
TCN – 7.5 minutes
TCC – 19.2 minutes
Cyano column
TCN – 4.0 minutes
TCC – 7.5 minutes

B-2.3 Procedure

B-2.3.1 *Standard Preparation* (*see* Note under **B-3.4**)

Weigh accurately 25 mg of triclosan (TCN) and 25 mg of TCC into a 100 ml volumetric flask and make up to volume with the mobile phase and mix well. Pipette 1.0 ml of this solution in a 50 ml volumetric flask and dilute with mobile phase. Final concentration of TCC and TCN is 250 µg/50 ml (5.0 ppm).

B-2.3.2 *Sample Preparation*

Weigh accurately 1g of homogenized sample into a 100-ml standard flask, and dilute to the mark with mobile phase. Pipette 10 ml of the supernatant liquid to a 50-ml volumetric flask, dilute with mobile phase, to the mark, and filter through 0.45 µm filter.

B-2.3.3 *Chromatography*

Equilibrate the column, maintained at a temperature of 30°C, with the mobile phase with a flow rate of 0.5 ml/min for *iso*-octane – *iso*-propanol mobile phase and 1.0 ml/min for hexane – *iso*-propanol mobile phase for 30 minutes. Set the wavelength at 280 nm. Inject 20µl of standard solution and then sample solutions. Measure area of the peaks of respective retention time for standard and sample.

B-2.4 Calculation

$$\text{TCN, percent by mass} = \frac{\text{Area of sample for TCN} \times \text{Concentration of standard TCN} \times 100}{\text{Area of standard TCN} \times \text{Concentration of sample}}$$

$$\text{TCC, percent by mass} = \frac{\text{Area of sample for TCC} \times \text{Concentration of standard TCC} \times 100}{\text{Area of standard TCC} \times \text{Concentration of sample}}$$

B-3 REVERSE PHASE

B-3.1 Reagents

B-3.1.1 *Methanol* – HPLC grade.

B-3.1.2 *Sodium Dihydrogen Phosphate Monohydrate* — Chemical grade.

B-3.1.3 *Standard TCC*

B-3.1.4 *Standard TCN (TCS)*

B-3.2 Apparatus

B-3.2.1 *Column* — Octyldimethylsilyl (C-DB).

Supercosil LC-8-DB — 15 cm × 4.6 mm. 5 µ.

B-3.2.2 *Mobile Phase*

MeOH/0.01 M Phosphate buffer 62:38 *v/v*

0.01M Phosphate buffer: Dissolve 1.38 g sodium dihydrogen phosphate monohydrate in 1 000 ml of distilled water. Prepare to *p*H 3.0 by 10 percent phosphate solution.

B-3.3 Procedure

B-3.3.1 *Standard Preparation* (*See* Note under **B-3.4**)

B-3-3.1.1 Weigh accurately about 90 mg TCN. Dissolve in methanol and make upto 1 000 ml volumetric flask with methanol.

B-3.3.1.2 Weigh about 110 mg of TCC, dissolve well with methanol, and make up the volume to 1 000 ml.

B-3.3.1.3 Accurately pipette 10 ml of the solution prepared in (*see* **B-3.3.1.1**) into the (*see* **B-3.3.1.2**) volumetric flask containing TCC. And make up to the volume with methanol. Then accurately pipette 5-ml of the solution into a 50-ml volumetric flask. Make upto the volume with methanol. Filter this standard solution through 0.45 µm filter.

B-3.3.2 *Sample Preparation*

Weigh accurately about 1.0 g of product, dissolve in methanol and make up to 100 ml in a volumetric flask with methanol. Filter this sample solution through 0.45 µm filter.

B-3.3.3 *HPLC Conditions*

Detector wavelength	:	280 nm
Column temperature	:	35°C
Flow rate	:	1.0 ml/min.
Injection volume	:	10 µl

Prepare the standard solution and the sample solution at the same time. Inject the standard solution three times and calculate the average of each ingredients peak count. Inject 10 µl the sample solution and determine each ingredients percentage by the calculation shown.

B-3.4 Calculations

$$\text{TCN percent by mass} = -\frac{M_s \times A_r \times F}{A_s \times M_t \times 100}$$

$$\text{TCC percent by mass} = -\frac{M_s \times A_r \times F}{A_s \times M_t \times 10}$$

where

M_s = Mass of the standard (g),

A_s = Averaged peak area of the standard,

M_t = Mass of the test sample (g),

A_r = Peak area of the test sample, and

F = Purity of standard (percent).

NOTE — Both TCC and TCN are photosensitive, hence standards should be freshly prepared.

ANNEX C

(Clause 4.2.2)

DETERMINATION OF CHLOROANILINE

C-1 PRINCIPLE

The chloroanilines are extracted from soap with dimethyl sulfoxide and diazotized with nitrous acid. The reaction products are then coupled with N-1-(naphthyl) ethylenediamine hydrochloride to produce coloured compounds which are estimated spectrophotometrically.

C-2 SAFETY PRECAUTIONS

Dimethyl sulfoxide (DMSO) is readily absorbed into the skin. Inhalation or skin penetration must be avoided.

DMSO should never be pipetted using mouth. Always use pipette bulb. The standard chloroanilines and N-1-(naphthyl) — ethylenediamine hydrochloride must not be allowed to come into contact with the skin. If they should, then wash the contaminated parts thoroughly with soap and water.

A supply of diluted sodium hypochlorite should be at hand at all times to deal with accidental spillages of chloraniline solution. Spillage on laboratory surface should be treated immediately with the sodium hypochlorite solution, followed by water.

C-3 REAGENTS

C-3.1 Dimethyl Sulphoxide (DMSO) — AR grade.

C-3.2 Hydrochloric Acid — Concentrated (specific gravity –1.18).

C-3.3 Sodium Nitrite — 0.4 percent *w/v* analytical grade, freshly prepared (aqueous).

C-3.4 Ammonium Sulphamate — 2 percent *w/v* solution freshly prepared (aqueous).

C-3.5 N-1-(naphthyl) Ethylene — 0.1 percent *w/v* solution diamine hydrochloride freshly prepared (aqueous).

C-3.6 *n*-Butanol — AR grade.

C-3.7 Sand — Acid purified 40-100 micron mesh.

C-3.8 Solvent Mixture

DMSO	5 Volumes
n-Butanol	2 Volumes
Distilled water	2 Volumes
Hydrochloric acid	1 Volume

Mix *n*-butanol, water and HCl. Cool the mixture and add DMSO.

C-3.9 4-Chloroaniline and 3, 4-Dichloroaniline — AR grade.

C-4 APPARATUS

C-4.1 Spectrophotometer — Suitable for use at 554 nm.

C-4.2 Cuvettes — Glass (matched pair) 10 mm.

C-4.3 Water Bath — Thermostatically controlled at 25°C.

C-4.4 Stop Watch

C-4.5 Standard Laboratory Glassware

C-4.6 Filter Paper — Whatman No. 541.

C-5 PROCEDURE

C-5.1 Preparation of Calibration Curve

C-5.1.1 Dissolve 0.349 8 g of 3, 4-dichloroaniline and 0.275 3 g of 4-chloroaniline in solvent mixture (*see* **C-2.8**) in a 250 ml amber volumetric flask. Dilute to mark with solvent mixture. 1 ml = 2:5 mg mixed chloroanilines (stock solution).

C-5.1.2 Dilute this stock solution with solvent mixture as given below:

a) Take 5 ml of stock solution and dilute it to 250 ml with solvent mixture.

   1 ml = 50 µg mixed chloroanilines.

b) Take 5 ml of the above solution [*see* **C-5.1.2**(a)] and further dilute to 250 ml with solvent mixture.

   1 ml = 1 µg mixed chloroanilines.

Use this solution for preparation of calibration curve.

Transfer using a burette 0, 1, 2, 5, 10, 20, 40 ml into 50 ml amber volumetric flasks.

C-5.1.3 From a burette, add sufficient solvent mixture to make total volume to 40-ml in each flask. The flasks are incubated in a water bath at 25°C for 20 min. After exactly 20 minutes, add 2-ml of reagent (*see* **C-3.3**) into each flask and return them to the water bath for exactly 10 min (measure with a stop watch).

Then add 2-ml of reagent (*see* **C-3.4**) into each flask and return them to the water bath for exactly 10 minutes. Swirl the flask occasionally.

Then add 2-ml of reagent (*see* **C-3.5**) into each flask and remove them from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 minutes. Measure absorbance at 554 nm against the blank solution as prepared in **C-5.1.4**.

C-5.1.4 *Preparation of Blank Solution*

Take 40 ml of solvent mixture in a 50 ml amber volumetric flask. Incubate the flask in a water bath at 25° C for 20 min. After exactly 20 min, add 2 ml of reagent (*see* **C-3.3**) into the flask and return it to the water bath for exactly 10 minutes. Then add 2 ml of reagent (*see* **C-3.4**) into the flask and return it to the water bath for exactly 10 minutes (Swirl the flask occasionally). Then add 2 ml of reagent (*see* **C-3.5**) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 minutes. Use this blank solution for preparation of calibration curve only.

C-5.1.5 Prepare a graph by plotting weight (µg) of chloroanilines contained in each 50 ml flask against absorbance. The linear calibration will pass through the origin/or determine the average absorbance (AA) of 1 µg of mixed chloroanilines by dividing sum of absorbances of all different aliquots of the standard by sum of µg of chloroanilines in all different aliquots of standard.

C-6 DETERMINATION OF CHLOROANILINES

C-6.1 Weigh to the nearest mg 3.0-3.5 g of finely grated soap add 10.0 - 15.0 g of acid purified sand. Transfer quantitatively the sample and the sand into a mortar and grind the mixture thoroughly with a pestle to give a homogenous mass. Transfer the mass to a previously weighed 250 ml flat bottom flask quantitatively and reweigh. Add DMSO (100 ml), stopper firmly and attach the flask to an automatic shaker. Shake for 1 h. Filter the DMSO extract through Whatman No. 541 into a 250 ml amber volumetric flask. Wash the flask and filter paper with small aliquots of DMSO. Allow the filtrate to drain completely, dilute to volume with DMSO and mix. Transfer 20 ml DMSO extract into a 50 ml amber volumetric flask. Add 20 ml of solvent mixture. The flask is incubated in a water bath at 25°C for 20 minutes. After exactly 20 minutes, add 2 ml of reagent (*see* **C-3.3**) into the flask and return it to the water bath for exactly 10 minutes (measure with a stop watch). Then add 2 ml of reagent (*see* **C-3.4**) into the flask and return it to the water bath for exactly 10 minutes (swirl the flask occasionally). Then add 2 ml of reagent (*see* **C-3.5**) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 minutes. Read the absorbance at 554 nm against blank (prepared as below).

C-6.2 Preparation of Blank

Prepare the blank solution by mixing 20 ml of DMSO extract of sample and 20 ml of solvent mixture in a 50 ml amber volumetric flask. Incubate the flask in a water bath at 25°C for 20 min. After exactly 20 minutes, add 2 ml of distilled water into the flask and return it to the water bath for exactly 10 minutes. Then

add 2 ml of reagent (*see* **C-3.4**) into the flask and return it to the water bath for exactly 10 minutes (swirl the flask occasionally). Then add 2 ml of reagent (*see* **C-3.5**) into the flask and remove it from the water bath. Dilute to volume with distilled water, mix and allow to stand for 30 min. Use this solution as a blank for reading sample only.

C-6.3 Deduce the amount of chloroanilines (μg) from the calibration graph curve.

NOTE — The determination must be completed in one day.

C-7 CALCULATIONS

C-7.1 Determine the amount of mixed chloroanilines in the aliquot of test solution from the calibration graph.

$$\text{Chloroaniline content (in ppm)} = \frac{250\left(M + M_1\right)M_3}{20 M_2 M}$$

where

M = mass in g of soap,

M_1 = mass in g of sand,

M_2 = mass in g of soap and sand transferred to the flask,

M_3 = mass (μg) of mixed chloroanilines found from calibration graph/or it can be calculated as given below:

$$M_3 = \frac{\text{Mass of the sample}}{\text{Average absorbance of 1 μg mixed chloroanilines } (AA)}$$

where

$$AA = \frac{\text{Sum of the OD of the standards}}{\text{Sum of concentration of standard chloroanilines in μg}}$$

$$\text{Weight of soap actually used, in } g = \frac{M_2 M}{(M + M_1)}$$

ANNEX D

(Foreword)

COMMITTEE COMPOSITION

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